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Fructose Consumption and Moderate Zinc Deficiency Influence Growth and Adipocyte Metabolism in Young Rats Prone to Adult-Onset Obesity

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Abstract The effects of low zinc, high fructose diet on growth and adipocyte metabolism were examined in rats. At 28 days of age, animals were assigned to diets either adequate in zinc (30 ppm) with water (AZW) or fructose solution (AZF), or low in zinc (5 ppm) with water (LZW) or fructose solution (LZF). Body weight and food and fructose solution intake were measured three times a week. Blood samples were collected at baseline, 4 weeks, and 8 weeks, and energy expenditure was measured. The rats were killed at 12 weeks. Adipocytes were cultured in medium containing C¹⁴-glucose and physiological insulin concentrations. The animals in the LZF group consumed less energy and gained less weight than the other groups. Serum zinc concentrations were lower in the LZF than the AZF group. Energy expenditure over a 24-h period did not differ between groups; however, the respiratory quotient in the fed state was higher in the groups consuming fructose solution than in those consuming water. The mesenteric adipocytes from the animals in the LZF group utilized more glucose. Thus, the addition of fructose to a LZ diet reduced energy intake and growth and altered adipocyte fuel metabolism in young growing rats.

Keywords Zinc · Fructose · Adipocyte · Growth · Insulin sensitivity

Introduction

The prevalence of obesity is increasing worldwide. This may be due, in part, to an increase in high-energy diets that are relatively low in micronutrients [1], including zinc. Fructose, in the form of high fructose corn syrup, is often part of high-energy, micronutrient-poor foods.

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Fructose consumption has increased by 30% between 1970 and 1990, and accounts for more than 20% of daily carbohydrate intake in the US [2]. Fructose is metabolized differently from glucose in several ways that may lead to increased adiposity. For example, glucose can cross the blood–brain barrier and influence the activity of populations of neurons involved in the regulation of food intake, whereas fructose does not readily cross the blood–brain barrier. This was demonstrated in an experiment in rats in which the animals' feeding behavior was stimulated with an intravenous dose of 2-deoxy-D-glucose (2-DG), a competitive inhibitor of glucose uptake. Infusion of glucose, but not fructose, into the jugular vein inhibited the 2-DG-induced feeding response [3]. Fructose and glucose metabolism differ in peripheral tissues as well. Glucose enters glycolysis before the rate-limiting phosphofructokinase step, whereas fructose enters afterward and is not subject to its regulation. Thus fructose, if consumed in large amounts, is more likely to proceed through glycolysis in the liver, providing glycerol and acetyl CoA substrates for triglyceride (TG) synthesis [4]. Also, glucose and fructose influence the hormones insulin and leptin differently. Glucose is the major dietary-derived substrate responsible for insulin secretion, and insulin-stimulated glucose uptake in the adipocyte is an important regulator of leptin production [5]. Fructose, however, does not elicit these key hormonal signals involved in long-term control of energy homeostasis [6].

Consumption of some important micronutrients tends to be low among Americans. Zinc is an example. Data from the third National Health and Nutrition Survey showed that 45% of Americans consumed less than 77% percent of the 1989 zinc RDA, a cutpoint considered to be a low zinc diet [7]. Low zinc intakes may be associated with increased risk of obesity. In studies of middle-aged [8] and young adults [9], plasma zinc concentrations were significantly lower in obese than in normal weight subjects. Plasma zinc was also lower in obese children, who had other indicators of impaired zinc status, including lower erythrocyte zinc concentrations [10].

Glucose intolerance is common among obese individuals, and zinc plays a role in regulating glucose metabolism. Compared with zinc-replete animals, zinc-deficient (ZD) rats had higher blood glucose values after intraperitoneal glucose administration [11, 12]. This may have been because of decreased insulin secretion [13] or sensitivity [11, 14]. Evidence from some human studies also suggests a relationship between zinc deficiency and impaired glucose intolerance [15] and indicates that zinc supplementation may improve glucose disposal [16, 17]. Recent animal studies have identified a zinc–leptin interaction. ZD rats have lower plasma leptin concentrations, adipocyte leptin mRNA, and leptin secretion from isolated adipocytes compared with pair-fed (PF) control animals [18, 19].

Because a low zinc, high fructose diet may alter the ability of insulin and leptin to regulate energy metabolism, we hypothesized that animals fed a high fructose, moderately low zinc diet would exhibit increased energy intake and fat gain. This hypothesis was tested by measuring (1) the efficiency of energy use for growth, (2) energy expenditure and carbohydrate oxidation, (3) serum insulin and leptin levels, and (4) adipocyte glucose utilization in rats that we have previously demonstrated are susceptible to adult-onset obesity and insulin resistance [20].

Methods

Animals and Diets

At weaning (21 days), 32 male Sprague–Dawley rats from the University of California Davis Nutrition Colony, derived from animals originally purchased from Charles River, were housed

individually in wire hanging cages in a temperature- and humidity-controlled room. We have previously demonstrated that this line of animals develops moderate obesity and insulin resistance between 1 and 3 months of age [20]. Lighting was controlled, with the 12-h dark cycle beginning at 7 PM. For a 1-week acclimatization period, all rats were provided ad libitum access to tap water and the zinc adequate diet. At 1 month of age, a baseline blood sample was drawn and then the rats were assigned to one of four dietary treatment groups for the 12-week study based on body weight. The four diet groups were adequate zinc (30 ppm) with water (AZW), adequate zinc with fructose solution (20% fructose in water) (AZF), low zinc (5 ppm) with water (LZW), and low zinc with fructose solution (LZF).

The animals were fed a semipurified diet ad libitum purchased from Research Diets (New Brunswick, NJ, USA). The diets contained 51% sucrose by weight and an additional 15.3% of carbohydrate primarily from cornstarch (Table 1). Egg white was the primary source of protein (20% by weight), and corn oil was the primary source of fat (5% by weight). The animals receiving water had ad libitum access to tap water. The animals receiving fructose were given fructose solution (20% by weight in distilled water) providing 0.74 kcal/g ad libitum flavored with Kool-aid™. To accurately assess energy intake from the fructose solution, the delivery system included a container to collect any spillage that could then be weighed and subtracted from total intake.

Body weight, food intake, and fructose solution intake were measured three times a week throughout the 12-week study. The animals were weighed to the nearest gram. The food bin, food spillage, fructose solution bottle, and fructose solution spillage were measured to the nearest 0.1 g. Water intake in the AZW and LZW groups was not measured.

Table 1 Composition of the Diets

	AZ (30 ppm)		LZ (5 ppm)		AZ (30 ppm)		LZ (5 ppm)	
	gm%	kcal%	gm%	kcal%	g/kg	kcal	g/kg	kcal
Macronutrient								
Protein	20.0	20.5	20.0	20.5				
Carbohydrate	66.3	68.0	66.3	68.0				
Fat	5.0	11.5	5.0	11.5				
Total	91.3	100.0	91.3	100.0				
kcal/gm	3.90		3.90					
Ingredient								
Egg Whites, Spray Dried					200	800	200	800
Corn Starch					150	600	150	600
Sucrose					502	2,010	503	2,010
Cellulose, BW200					50	0	50	0
Corn Oil					50	450	50	450
Mineral Mix S10001					35	0	35	0
Vitamin Mix V10001					10	40	10	40
Choline Bitartrate					2	0	2	0
Biotin, 1%					0.4	0	0.4	0
Zinc Carbonate, 52.1% Zinc					0.056	0	0.008	0
Total					1,000	3,900	1,000	3,900

Blood Sampling and Analysis

At 28 days before assignment to the study diets a nonfasting baseline blood sample was collected from the tail of each animal. The animals were restrained, their tails cut at the tip (~1–2 mm), and 500 μ l of blood was collected into a 1.5-ml vial. The blood samples were kept on ice for a maximum of 1 h until centrifugation at 3,000 rpm for 10 min. The serum was then transferred to another 1.5-ml vial and frozen until analyzed for zinc. Three days later, a second blood sample was collected in a similar manner, except the animals were fasted for 14 h beforehand. At the beginning of this blood draw a small amount (~20 μ l) of blood was collected in a separate vial for the analysis of glucose and free fatty acids (FFAs). This blood sample was collected first to reduce the influence of stress from handling on these metabolites. This sample was also kept on ice, then centrifuged at 12,000 rpm for 5 min and the serum transferred to another vial and frozen. Nonfasting and fasting tail blood samples were collected at 4 and 8 weeks of the study (8 and 12 weeks of age) (Table 2).

Serum glucose was measured on an Analox glucose analyzer (model GM7 MicroStat, Analox, Lunenburg, MA 01462, USA). Serum TGs and FFAs were measured using an enzymatic assay from Wako Chemicals (Richmond, VA 23237, USA). Serum insulin was measured using an ELISA assay from Linco Research (St. Charles, MO 63304, USA). Serum leptin and adiponectin were measured with RIA kits from Linco. Serum zinc was measured using inductively coupled argon plasma–atomic emission spectrophotometry (ICP-AES) after wet ashing with 1 N HNO₃.

Measurement of Energy Expenditure and Respiratory Quotient

At 4 and 8 weeks of the study, the respiratory quotient (RQ) and energy expenditure of the animals were measured by indirect calorimetry using the Integra ME System from AccuScan Instruments, Inc. (Columbus, OH 43228, USA) (Table 2). The animals were transferred to the 16.5×16.5×12" Plexiglas chambers, one animal per chamber, for the 3-day measurement. The four chambers and the air reference line were connected to a flow controller/channelyzer that allowed flow rate adjustments and sequential channeling of the airflow through the CO₂ and O₂ analyzer. The system software included a calibration program for the CO₂ and O₂ analyzer and data collection and analysis programs. After a 24-h acclimatization period, RQ was determined by the ratio of CO₂ produced over O₂ consumed and energy expenditure was calculated from the following: $(4.33 + 0.67 \times \text{RQ}) \times \text{O}_2 \times \text{animal weight (in grams)} \times 60$ [21].

Table 2 Study Schedule

Study Week	Rat Age (Week)	Procedure
–1	3	Start control diet (acclimatization)
0	4	Tail blood collection (nonfasting and fasting); start experiment
4	8	Tail blood collection, energy expenditure, and RQ measurements
8	12	Tail blood collection, energy expenditure, and RQ measurements
12	16	Kill animals: fat depot dissection and adipocyte culture

Fat Depot Removal and Analysis

After an overnight fast at study week 12 (age 16 weeks), the rats were anesthetized with halothane and blood was collected by cardiac puncture and the animals were killed by opening the diaphragm. The epididymal and mesenteric fat depots were then dissected free and weighed to the nearest 0.01 g. A sample was taken from each of the two fat depots, frozen in liquid nitrogen, and stored in a -80°C freezer until analyzed for zinc. Tissue zinc concentrations were determined by ICP-AES after wet ashing with 1 N HNO_3 .

Adipocyte Culture Studies

Fat depot samples weighing between 3–4 g were taken for adipocyte culture studies. They were first minced in HEPES/phosphate buffer (pH 7.4; 5 mM D-glucose, 2% bovine serum albumin, 135 mM NaCl, 2.2 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.25 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.45 mM KH_2PO_4 , 2.17 mM Na_2HPO_4 , and 10 mM HEPES) and digested with collagenase (1.25 mg/ml per 0.5 g tissue) in a 37°C water bath with gentle shaking for 30 min. The resulting cell suspension was then diluted in more HEPES–phosphate buffer and the isolated adipocytes were separated from undigested tissue by filtration through a 400- μm nylon mesh and washed three times. Isolated adipocytes were then resuspended in Dulbecco's Modified Eagle Medium supplemented with 1% fetal bovine serum and incubated for 40 min at 37°C . The cells (150 ml of 2:1 ratio of packed cells to medium) were plated on 500 ml of a collagen matrix (Vitrogen 100, Cohesion Technologies, Palo Alto, CA 94303, USA) in six-well culture plates. After 50 min of incubation at 37°C , 2 ml of the culture media containing 0, 0.48, or 1.6 nM of insulin and ^{14}C radiolabeled glucose (0.01 or 0.02 $\mu\text{Ci}/\text{ml}$) was added. The cells were kept in an incubator at 37°C in 5% CO_2 for 96 h. Three hundred microliters of the samples of the media were collected at 24, 48, 72, and 96 h; the media was replaced after the first three samples were taken. The samples were frozen and later analyzed for glucose and lactate with a YSI glucose analyzer (Model 2300, Yellow Springs Instruments, Yellow Springs, OH 45387, USA).

After 96 h, the incorporation of ^{14}C -glucose into lipid was measured by removing all of the media from the wells, adding 2 ml of methanol to the wells, scraping the cells from the wells, and transferring the solution to 50 ml glass tubes with screw tops. This process was repeated to ensure complete transfer. Ten milliliters of chloroform was added to the tubes and the tubes were capped. After 24 h, water was added to the tubes. The water–methanol layer was aspirated off, and 1 ml of the samples of the chloroform–lipid layer were transferred to scintillation vials and counted for beta radioactivity (disintegrations per minute [dpm]). The amount of glucose incorporated into lipid was calculated as $[(\text{dpm extracted in 1 ml of chloroform})/(\text{total glucose})/(\text{total dpm})] \times 10 \text{ ml of chloroform}$.

Table 3 Intakes of Food, Fructose, Energy, and Zinc

Treatment Group	Food (kcal/day)	Fructose (Food + Liquid) (kcal/day)	Total Energy (Food + Liquid) (kcal/day)	Zinc (mg/day)
AZW	102 \pm 6 ^a	24 \pm 1 ^a	102 \pm 6 ^a	0.78 \pm 0.05 ^a
LZW	95 \pm 3 ^a	23 \pm 1 ^a	95 \pm 3 ^{ab}	0.12 \pm 0.01 ^c
AZF	77 \pm 4 ^b	44 \pm 3 ^b	102 \pm 3 ^a	0.59 \pm 0.03 ^b
LZF	70 \pm 4 ^b	33 \pm 2 ^c	86 \pm 4 ^b	0.09 \pm 0.01 ^c
<i>p</i> value	<0.0001	<0.0001	0.03	<0.0001

Values within a column with different superscripted letters are significantly different, $p < 0.05$.

Table 4 Growth Rate, Ending Body Weight, and Energy Efficiency

Treatment Group	Growth Rate (g/day)	Body Weight at Week 11 (g)	FER (mg kcal ⁻¹ day ⁻¹) ^a
AZW	5.89±0.37 ^a	569±27 ^a	57.4±1.9
LZW	5.55±0.19 ^{ab}	547±15 ^{ab}	58.3±1.5
AZF	5.82±0.21 ^a	569±16 ^a	61.2±1.7
LZF	4.71±0.25 ^b	484±21 ^b	55.0±1.3
<i>p</i> value	0.01	0.02	0.07

Values within a column with different superscripted letters are significantly different, $p < 0.05$.

^a FER=growth (mg)/energy intake (kcal) per day.

Statistical Methods

All group comparisons were done using one-way analysis of variance (ANOVA), and post hoc testing was done using Tukey's test. Values are reported as mean±SEM. Differences were considered significant at a p value ≤ 0.05 .

Results

Food Intake and Growth Access to the 20% fructose solution for the two fructose solution groups (AZF and LZF) lowered their intake of solid food significantly (Table 3). However, total energy intake was lower the LZF animals than the other groups. The calculated zinc intakes of the two LZ groups were significantly less than the AZ groups.

The growth rate and final body weights of the LZF, but not the LZW group, were approximately 20 and 15% lower, respectively, than that of the two AZ groups ($p < 0.02$). In addition, the LZF group tended to have a decreased food efficiency ratio (FER), gaining less weight for the amount of calories consumed than the other groups ($p = 0.07$) (Table 4).

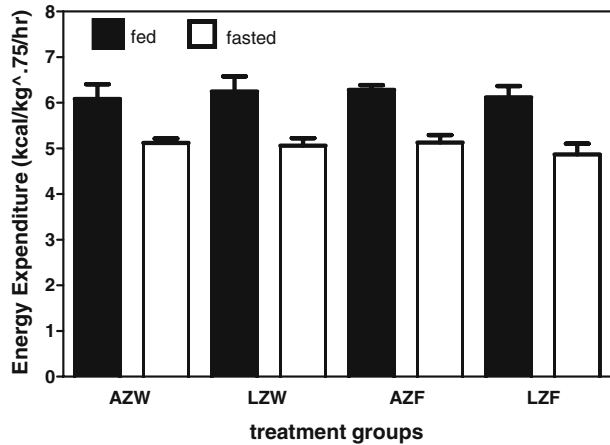
Serum and Fat Depot Zinc Concentrations Serum zinc concentrations were not reduced in animals fed a LZ diet with water in comparison to the two AZ diet groups. However, the serum zinc concentrations were significantly ($p = 0.04$) lower in the LZF group, possibly because total food intake and, therefore, zinc intakes were lowest in the LZF group (Table 5). The mesenteric and epididymal fat depot weights were not different between treatment groups. There were also no significant differences in the total amount of zinc or zinc concentrations in the mesenteric fat depots (Table 5).

Table 5 Serum Zinc at 12 Weeks, Fat Depot Weights, and Total Zinc Content and Zinc Concentration per Gram Fat Depot of the Mesenteric Fat Depots

Treatment Group	Serum Zinc Concentration (mg/dl)	Mesenteric Fat Depot Weight (g)	Epididymal Fat Depot Weight (g)	Total Zinc Mesenteric Fat Depot (μg)	Zinc Concentration Mesenteric Fat Depot (μg/g)
AZW	1.07±0.06 ^{ab}	5.01±0.56	8.44±1.04	445±55	95.1±14.4
LZW	1.10±0.06 ^{ab}	6.02±0.92	8.81±1.10	504±80	83.4±10.9
AZF	1.14±0.07 ^a	6.44±0.80	10.17±1.24	387±49	64.9±10.4
LZF	0.90±0.05 ^b	4.16±0.82	7.07±1.44	368±74	89.9±8.6
<i>p</i> value	0.04	0.19	0.37	0.46	0.25

Values within a column with different superscripted letters are significantly different, $p < 0.05$.

Fig. 1 Energy expenditure (kcal $\text{kg}^{-0.75} \text{h}^{-1}$) from 5–7 AM after a fed (black) and a fasted (white) dark cycle at the second time point (study week 8)



Energy Expenditure and Respiratory Quotient As expected, the energy expenditure at the end of the dark cycle (5–7 AM) when the animals had access to food (“fed”) was higher than at the end of the subsequent dark cycle when their food had been removed 12 h before (“fasted”) (Fig. 1). Energy expenditure did not differ between treatment groups in either the fed or fasted states. RQ did not differ by group in the fasted state; however, in the fed state RQ was higher in the AZF and LZF groups (Fig. 2). As expected, increased carbohydrate intake in the fructose groups resulted in increased carbohydrate oxidation.

Serum Glucose, Lipid, and Hormone Concentrations Fasting concentrations of glucose, FFA, or TGs did not differ among the four groups (Table 6). There were also no effects of the dietary treatments on serum insulin, leptin, and adiponectin concentrations. However, several of these parameters changed over the course of the experiment. Serum glucose concentrations increased and FFA concentrations declined over the 8-week experiment, whereas insulin and leptin increased and adiponectin decreased (Table 6).

Fig. 2 Respiratory quotient from 5–7 AM after a fed (black) and a fasted (white) dark cycle at the second time point (study week 8). Within fed and fasted series, columns with different superscripts are significantly different; ANOVA p value < 0.005

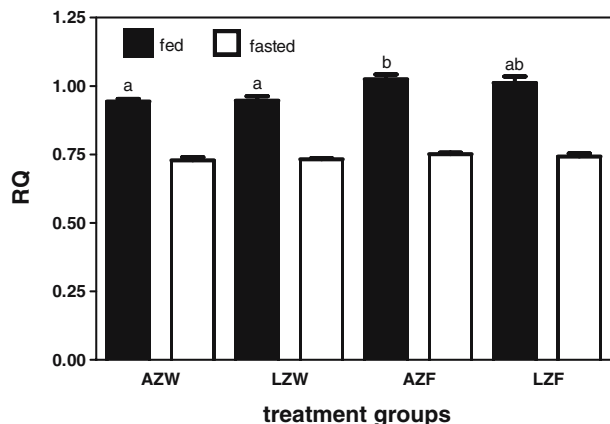


Table 6 Substrate and Hormone Concentrations at Baseline and Study week 8

	Group	Baseline	4 Week	8 Week	<i>p</i> Value
Glucose (mg/dl)	AZW	91.2±4.3	87.5±3.6	101.3±4.2	0.15
	LZW	82.9±2.2	83.2±5.4	93.2±3.9 ^a	0.05
	AZF	85.2±3.2	91.2±1.7	96.7±5.1	0.09
	LZF	79.0±3.8	91.0±3.9	96.9±5.8 ^a	<0.01
FFA (mEq/l)	AZW	1.35±0.10	1.26±0.08	1.03±0.08 ^b	<0.01
	LZW	1.31±0.08	1.38±0.13	1.01±0.08 ^b	0.04
	AZF	1.37±0.11	1.23±0.05	1.19±0.08	0.23
	LZF	1.36±0.07	1.30±0.07	1.14±0.13	0.11
TG (mg/dl)	AZW	67.1±10.2	51.3±6.3	63.9±10.0	0.73
	LZW	86.9±12.9	59.0±10.2	58.7±9.4	0.18
	AZF	82.0±16.5	74.9±8.0	76.8±10.9	0.80
	LZF	81.5±6.4	61.1±8.4	74.6±11.3	0.62
Insulin (ng/ml)	AZW	1.12±0.16	1.37±0.15	2.04±0.36 ^a	0.02
	LZW	1.10±0.22	1.53±0.24	1.98±0.42	0.11
	AZF	1.10±0.10	2.48±0.77	2.11±0.22 ^a	<0.01
	LZF	0.78±0.07	1.74±0.28	1.81±0.38 ^a	0.04
Leptin (ng/ml)	AZW	0.68±0.13	2.22±0.45	2.87±0.56 ^a	<0.01
	LZW	0.69±0.08	2.35±0.79	3.66±0.50 ^a	<0.01
	AZF	0.56±0.07	3.14±0.90	4.91±0.71 ^a	<0.01
	LZF	0.66±0.16	2.03±0.30	3.76±0.93 ^a	<0.01
Adiponectin (μg/ml)	AZW	3.73±0.20	3.08±0.23	3.01±0.29	0.08
	LZW	3.87±0.24	3.42±0.20	3.01±0.11 ^b	<0.01
	AZF	3.64±0.29	2.92±0.23	2.86±0.26 ^b	0.02
	LZF	3.78±0.25	3.75±0.12	3.08±0.12 ^b	0.03

^a Indicates 8-week value significantly higher than baseline by paired *t* test, *p*<0.05

^b Indicates 8-week value significantly lower than baseline by paired *t* test, *p*<0.05

Adipocyte Metabolism In comparison to the other groups, adipocytes isolated from the mesenteric fat pads of the LZF animals utilized significantly more glucose in both the absence and presence of insulin (Fig. 3). In addition, adipocytes from these animals incorporated more glucose into lipid on an absolute basis (data not shown). However, on a percentage basis, glucose incorporation into lipid was not different between groups at any

Fig. 3 Glucose utilization over 96 h by adipocytes isolated from the mesenteric depot in the absence of insulin and in the presence of 0.48 and 1.6 nM insulin. An asterisk (*) indicates that glucose utilization in the LZF group is greater than at least one of the other groups; ANOVA *p* value=0.03 or less

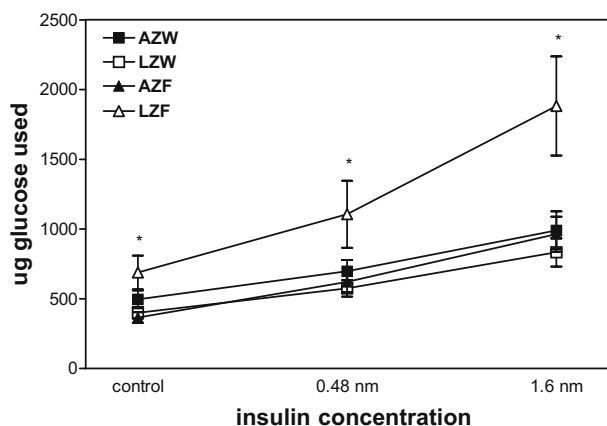
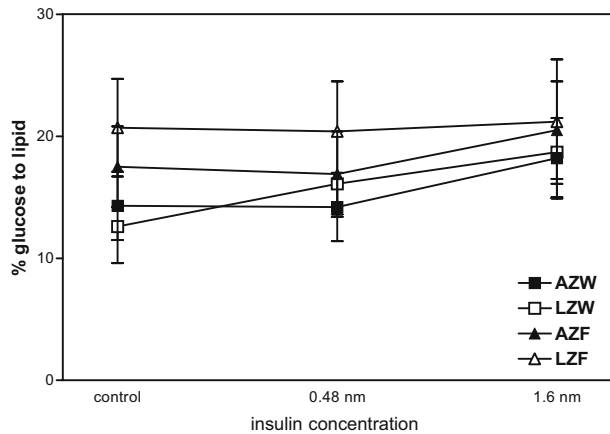


Fig. 4 Percentage of glucose incorporated into lipid over 96 h in adipocytes isolated from the mesenteric fat depot in the absence of insulin and the presence of 0.48 and 1.6 nM insulin



insulin concentration (Fig. 4). There were no significant differences between groups in lactate production or the proportion of glucose metabolized to lactate. Parameters of adipocyte metabolism in adipocytes isolated from the epididymal depot did not differ between treatment groups (data not shown).

Discussion

The results of this study do not provide support for the hypothesis that a low zinc diet accompanied by dietary fructose increases energy intake resulting in increased weight gain in rats prone to adult-onset obesity. In fact, the rats in the LZF group consumed the least amount of energy, had the lowest body weights, and tended to have lower FERs relative to the AZF group. The LZF, but not the LZW group, had lower serum zinc concentrations than the AZ groups. There were no significant differences in serum leptin and insulin concentrations between groups, nor were there differences in serum adiponectin, glucose, TGs, or FFAs. Zinc status also did not affect energy expenditure, although RQ tended to be increased in the fed state in the animals consuming the fructose solutions. Adipocytes from the LZF group appeared to be more insulin-sensitive, utilizing more glucose in the presence of physiological concentrations of insulin.

Anorexia and weight loss are well known as symptoms of severe zinc deficiency; however, moderate zinc restriction usually does not reduce energy intake and body weight [22, 23]. In this study, the addition of a 20% fructose solution decreased food intake and body weight in the LZ group. This may be because the low zinc diet reduced the preference for fructose solution and food. Food intake of the LZF group tended to be lower than that of the AZF group. The carbohydrate content of the study diet was high, 66%. Previous studies have reported that the preference for carbohydrate relative to other macronutrients declines with low zinc intakes [24–26], and fructose appears to be the least preferred carbohydrate [27, 28].

A high fructose intake along with a low zinc diet also tended to decrease the efficiency with which food was used for growth; the FER was lower in the LZF group than the other three groups, but this decline did not reach significance ($p=0.07$). Previous studies of severe zinc deficiency (1 ppm) have shown a decrease in FER [29, 30]. However, in a study

of high sucrose diets, a moderately low zinc intake tended to increase FER [23]. This is in contrast the results of the present study, which suggest that fructose, added to a high sucrose diet, along with marginal zinc intakes, decreases the efficiency with which dietary energy is used for growth.

In addition to growing less, the LZF animals also had lower levels of serum zinc. Serum zinc concentrations did not decline in the LZW group or in the AZF group. Thus, it appeared to be the combination of a low zinc/high fructose intake that altered serum zinc concentrations. Total zinc intake was the lowest in the LZF group, but it was also reduced in the LZW group. Others have reported that a moderately low zinc intake (8 ppm), similar to that in our study (5 ppm), did not lower serum zinc in rats given water ad libitum [23]. Because fructose was shown to affect food intake in combination with zinc deficiency, Smith et al. [31] studied the effect of severe zinc deficiency on serum zinc concentrations; they found that the levels declined regardless of whether the carbohydrate source was fructose or starch.

Zinc intakes did not alter the energy expenditures of the animals provided with either the fructose solution or water, whereas consumption of the fructose solutions increased RQ in the fed state in both AZ and LZ animals. This differs from previously reported effects of severe zinc deficiency on metabolic rate. Both metabolic rate and RQ have been reported to decline in severely ZD rats and their PF counterparts [21, 30], suggesting that the effect is because of lower food intakes rather than zinc deficiency. However, locomotor activity declined in the ZD group, but not the PF group [21]. The authors concluded that activity energy expenditure is lowered by zinc deficiency, whereas other components of energy expenditure are not.

There were no differences in circulating substrate (glucose, FFA, and TG) or hormone (insulin, leptin, and adiponectin) concentrations between the four treatment groups. Hall et al. [22] also reported that a marginal intake of zinc (7 ppm) did not affect circulating insulin or glucose concentrations compared to controls, whereas a very low zinc intake increased serum glucose and reduced serum insulin concentrations in rats. In our study, differences in substrate and hormone concentrations were only observed as an effect of increasing age. In all four groups, serum glucose concentrations increased and FFA levels declined from baseline to 8 weeks. This shift was concurrent with a decline in adiponectin and an increase in insulin and leptin. Whereas the increase in glucose, insulin, and leptin and decrease in adiponectin are likely related to the animals' aging, FFAs usually increase with age rather than decrease. One explanation might be that FFAs, as a metabolite especially sensitive to stress, increased more at the first time point than the subsequent ones as the animals became more conditioned to handling and the blood collection procedure.

The increase of insulin sensitivity in adipocytes isolated from the mesenteric fat depot from the LZF group was unexpected because several other groups have reported that zinc deficiency decreases insulin sensitivity [11–14, 32]. Different methods were used to assess insulin sensitivity in those studies. This is the first study to examine the effect of zinc status on glucose utilization in mesenteric adipocytes. Previous studies utilized glucose tolerance tests, circulating insulin levels, or euglycemic hyperinsulinemic clamps to evaluate whole body insulin sensitivity. Because the size of the mesenteric fat pad is inversely related to insulin sensitivity [33, 34], we hypothesize that the increased insulin sensitivity in the adipocytes isolated from the LZF animals was related to their smaller fat depot size and, possibly, smaller adipocytes.

Conclusions regarding the changes in body fat and adipocyte metabolism in this study are limited by the observation that the animals consuming these semipurified, mineral-controlled diets consumed less energy compared with animals fed with standard rat chow

(data not shown). The four experimental groups consumed from 86 to 102 kcal/day, whereas chow-fed animals consumed 122 ± 2 kcal/day. The basal semipurified diet contained close to 50% sucrose by weight. Thus, the two groups provided with water had diets containing approximately 25% fructose by energy; and animals provided with the fructose solutions consumed around 40% fructose by energy from food and liquid. A larger difference in fructose intakes between the two groups might have induced metabolic adjustments and shifts in energy intakes and growth. Future studies need to be performed using mineral-controlled diets that do not contain sucrose and thereby allow larger differences of fructose intake to be studied, and with PF groups to differentiate the effects of low zinc status from the effects of high fructose intake.

In conclusion, a high fructose/moderately low zinc diet decreased energy intake, energy efficiency, and the overall rate of growth. The reduction in food intake was associated with a decline in serum zinc concentrations. However, circulating levels of metabolic substrates and hormones were not altered by the low zinc/high fructose diet. There were no significant effects of the diets on the size of the fat depots although the rate of glucose utilization in the mesenteric adipocytes was increased. Together, these data suggest that the combination of a high fructose diet with low zinc intakes alters energy utilization. Further research is needed, however, to investigate the mechanisms involved in these effects.

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